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Research Triangle Park, NC

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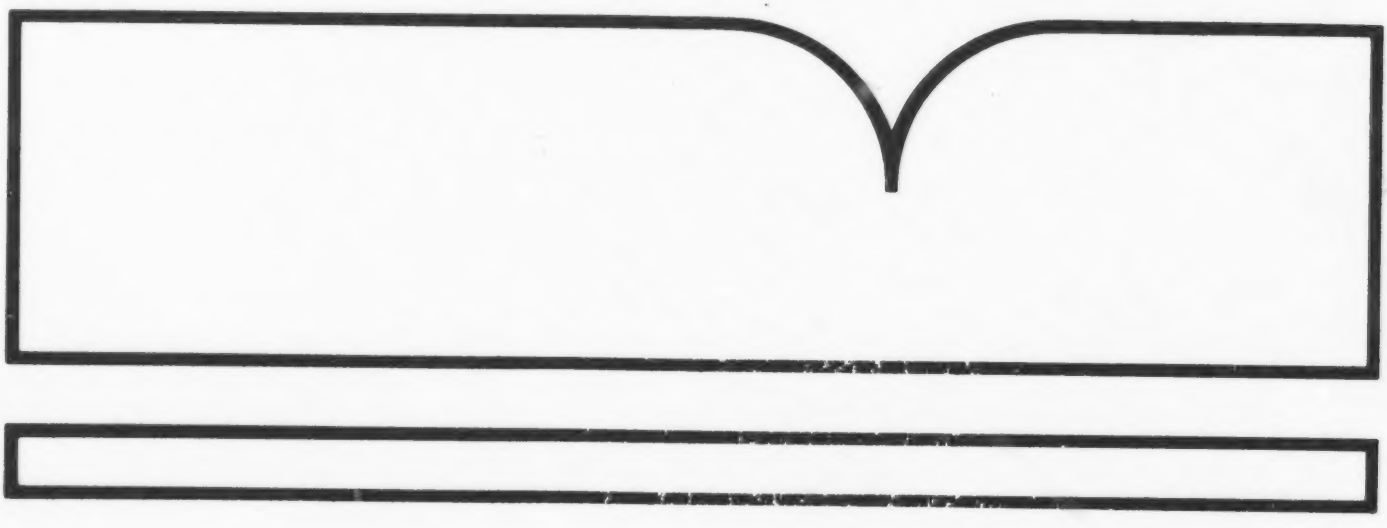

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16. ABSTRACT

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JOURNAL ARTICLE

Lethality in Mice and Rats Exposed to 2450 MHz Circularly Polarized Microwaves as a Function of Exposure Duration and Environmental Factors†

E. Berman,* J. B. Kinn, J. Ali, H. B. Carter and B. Rehnberg
Experimental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, USA

A. G. Stead
Biometry Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, USA

Key words: lethality; rodent; RF radiation.

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INTRODUCTION

It has proven to be a rather difficult and elusive task to confidently relate results of studies of radiofrequency radiation (RF) in laboratory animals. This is partly because of the interplay of observed effects with the degree of influence of exposure variables (frequency of radiation, field intensity, exposure duration, etc.), environmental conditions (temperature, humidity, air velocity), species size and other experimental parameters. To understand better this interplay, we have chosen a conventional test of biological response, the LD_{50}/24 h, and examined the influence which field intensity, exposure duration, environmental temperature and relative humidity may simultaneously have on incidence of death in the mouse and rat.

EXPERIMENTAL

Animals

and a 12 h : 12 h light-dark cycle. On the morning of exposure, groups of 16 mice or 6 rats were assigned to the experiment on the basis of body mass (range: 25–38 g, mean ± SD: 26.5 g ± 5.6% for mice; range: 385–430 g, mean ± SD: 408 g ± 5.6% for rats). The experimental design included combinations of independent variables of exposure (1 or 4 h), environmental temperature (20 or 30 °C), environmental relative humidity (35 or 80%), incident power density (10, 20, 50 or 75 mW cm^{-2}), and species (mice or rats). The schedule of treatment was randomized; for logistic reasons, mice were treated first, comparable 1- and 4-h exposures occurred on the same day, and substitutions were made when electrical or environmental conditions could not be met. Exposures were performed on Tuesdays to Fridays, between 09.00 and 15.00, with no prior adaptation to exposure conditions.

Just before exposure, mice assigned that morning were caged in clear polycarbonate containers, 1.5-mm thick and 6 cm on a side, drilled with 6–8 ventilation holes in each side and the top;[1] rats were caged in slatted acrylic plastic containers 8 cm high, 10 cm wide and 17.5 cm long.[2] The animals were then treated with one of 32 possible combina-

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EXPERIMENTAL

Animals

Male CD-1 mice and male CD rats were used in this study. When animals were received from the supplier (Charles River Laboratories, Kingston, NY), four mice or two rats were maintained by being caged together in shoe-box type cages with pine-shaving bedding, food and water *ad libitum*, and a 12 h : 12 h light-dark cycle. On the morning of exposure, groups of 16 mice or 6 rats were assigned to the experiment on the basis of body mass (range: 25–38 g, mean ± SD: 26.5 g ± 5.6% for mice; range: 385–430 g, mean ± SD: 408 g ± 5.6% for rats). The experimental design included combinations of independent variables of exposure (1 or 4 h), environmental temperature (20 or 30 °C), environmental relative humidity (35 or 80%), incident power density (10, 20, 50 or 75 mW cm^{-2}), and species (mice or rats). The schedule of treatment was randomized; for logistic reasons, mice were treated first, comparable 1- and 4-h exposures occurred on the same day, and substitutions were made when electrical or environmental conditions could not be met. Exposures were performed on Tuesdays to Fridays, between 09.00 and 15.00, with no prior adaptation to exposure conditions.

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Microwave exposure system

The four field intensities used in this study (10, 25, 50 and 75 mW cm^{-2}) are referred to as the nominal power density

† This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial producers does not constitute endorsement or recommendation for use.

* Author to whom correspondence should be addressed.

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(NPD). These exposure values were arrived at just before each animal exposure by placing the three-dimensional dipole probe (described below) in the center of the field. The power output was then adjusted to read the specific NPD on the digital display of the dipole probe. NPD also refers to the power density values resulting from the statistical analysis.

The microwave exposure system (Fig. 1) is a free-field microwave anechoic chamber type facility operating at 2.45 GHz.[3] The system emits circularly polarized radiation using a 9.5 dB antenna (Model 24HC001; Nurad, Inc., Baltimore, MD) mounted close to the center of the anechoic chamber ceiling. The microwave radiation propagates downward, while the electric and magnetic field vectors rotate in a horizontal plane. The interior dimensions of the anechoic chamber are 2.7 x 2.7 x 2.6 m with floor blocks made of 9-inch-cone structural absorber (Model RFP-9; Microwave Control Products, Winchester, MA) and walls and ceiling of Model FFP-5 5-inch-cone pyramidal RF absorber, except in areas of direct antenna radiation, where Model FFP-8 8-inch-cone absorbers were used. This system shares its anechoic chamber and environmental control system with an X-band exposure facility which has been described elsewhere.[3,4]

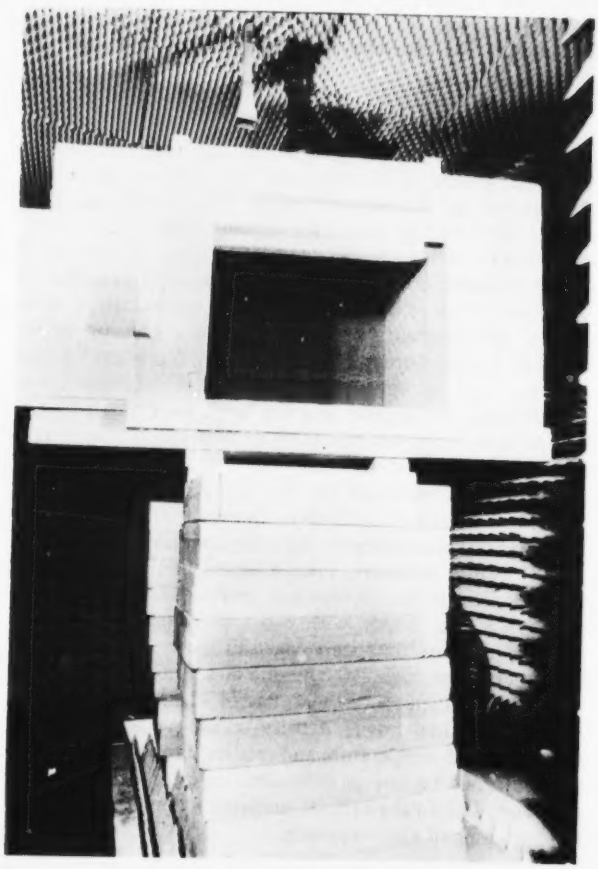

Figure 1. Photograph of interior of the anechoic chamber. The foamed styrene environmental chamber, on its foam styrene support blocks, is open. The horn of the circularly polarized waveguide is the round, dark guide immediately above the environmental chamber.

Available transmitted power is 1 kW CW, permitting a maximum radiated power density of 40 mW cm^{-2} at a distance of 130 cm from the antenna. The system has amplitude stabilization to minimize power line and temperature-induced drifts in RF radiated power.

Biological subjects were placed in a foamed polystyrene animal exposure chamber with interior dimensions 76.2 x 76.2 x 61 cm wide. This chamber is constantly flushed with environmentally controlled air. The metal-free ducting in the anechoic chamber is made of a neoprene-coated nylon fabric. The animal exposure chamber is also equipped with fiber optics 'house lights'. The lights were on during the exposures. A similar sham exposure chamber is located adjacent to the anechoic chamber.

Environmentally controlled air is fed through insulated ducts to the animal exposure chamber and sham chamber in parallel from a specially designed environmental control system[4] located adjacent to the anechoic chamber, and then recirculated. Range of temperature control is from −10 °C to +40 °C. The range of humidity control is from 20% to 80%. Air inlet and outlet temperatures are displayed, as well as relative humidity (%). Both temperature and relative humidity are regulated using three-mode proportional controllers. Average air velocity in the sham and animal exposure chambers was 12 m min^{-1}.

Equivalent plane-wave power density is measured using a three-element orthogonal dipole electric (E) field probe,[5] terminated with Schottky barrier diodes (HP model 5082-2800) at the gap between the dipole wing elements. The overall size of the tip of the dipole probe is 3 cm. The typical dipole response is a DC voltage which is non-linear with power density. A linearizer circuit using a digital table look-up technique was designed to provide a convenient digital display of total equivalent-plane wave incident power density. The probe and linearizer have a dynamic range of 10 μW cm^{-2} to 99.9 mW cm^{-2}.

The E-field was measured at 221 points equally spaced 4 cm apart on a 17 x 13 grid within the 76 x 62 cm exposure chamber. These values were used as data points in a CALCOMP GPCP (General Purpose Contouring Program, California Computer Products, Inc., Anaheim, CA) plotting program to produce a contour map of the E-field (Fig. 2). Using these contour maps as a guide to avoid areas of high gradients and relative maxima and minima, we designed several animal arrays using cage-spacing greater than a quarter wavelength. The uniformity of specific absorption rate (SAR) in each of these arrays was tested using water loads simulating animals. The arrays selected (Fig. 3) for this study had the smallest positional variation of SAR. Multiple measurements of SAR for each position in these two arrays were determined with twin-well calorimetry using animal carcasses, when all other positions during exposure were filled with live animals. The mean SAR for all positions had an overall average and variation of 6.94 W kg^{-1} ± 26% for mice and 3.5 W kg^{-1} ± 48% for rats. The overall average and variation of body weight for these animals were 26.1 g ± 4% for mice and 399 g ± 4% for rats.

Statistical methodology

To consider the effects of NPD, exposure duration, environmental temperature and relative humidity, and species on the animals' mortality experience, a 1/2 replicate of a 4×2^4 factorial experiment[6] was chosen. With 16 mice or

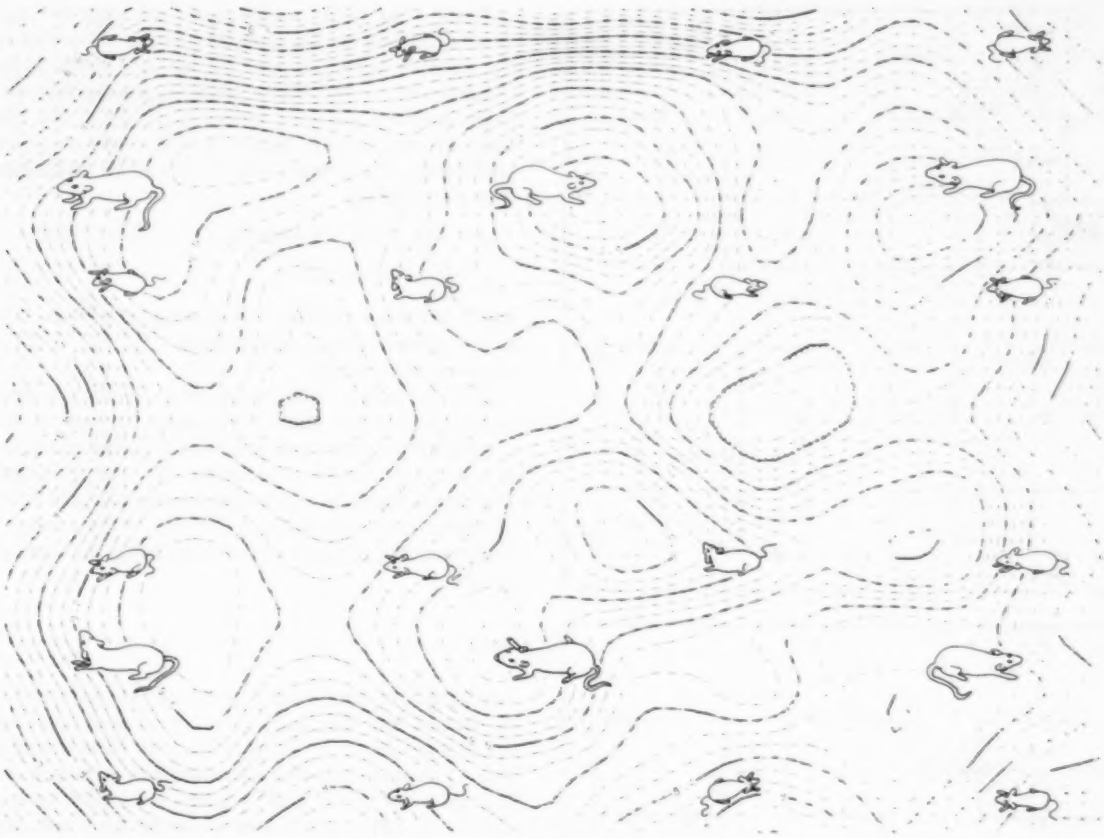

Figure 2. Contour map of the free field power density of the circularly polarized 2450-MHz source. Values are relative. The sketches of animals show the location of 16 mice or 6 rats.

6 rats at each treatment combination, a total of 512 mice and 192 rats were studied.

Probit analysis[7] has often been employed in the examination of binary response data generated from groups of subjects tested at various dose levels. The probability (P) of a subject 'responding' at dose X is described as:

$$P = \phi(\beta_0 + \beta_1 X) = \int_{-\infty}^{\beta_0 + \beta_1 X} (\exp(-Z^2/2)/\sqrt{2\pi})\, dz \qquad (1)$$

where ϕ is the unit normal cumulative distribution function, X is the dose or log dose, Z is the standard normal variate, dz is the differential of Z, β_0 is the intercept and β_1 is the slope of the probit regression line.

Until recently, the extension of this type of analysis to handle probits involving linear combinations of more than one independent variable was seldom undertaken, due to the cumbersome computations necessary to arrive at estimates with good properties. However, the advent of sophisticated statistical software such as the General Linear Interactive Modeling (GLIM) package[8] has made such analyses relatively simple. Briefly, this means that Eqn (1) can be extended to

$$P = \phi(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_n X_n) \qquad (2)$$

where x_i may represent continuous or classification (dummy) variables or interactions between variables. Thus, the probit model given by Eqn (2) can be used within either an analysis of variance or multiple regression framework. Tests of the contributions of the various model terms are performed by examining χ^2 statistics derived from likelihood ratios estimated by fitting reduced models.

Applying the analysis of variance probit model, we first determined which effects were contributing significantly to mortality rate differences. Upon isolating these effects, a multiple regression probit model was fit to estimate the coefficients (β) of the selected variables when viewed quantitatively. With the latter model, we can predict the proportion of animals dying for various combinations of the study variables and can also estimate LD_{50}'s at different levels of the non-dose variables. Due to computer memory restraints, separate models were developed for each species.

RESULTS

Table 1 contains the incidences of dead mice and rats for each combination of NPD, exposure duration, and environmental temperature and relative humidity used in the study. The number of deaths represent those animals that died by the end of the exposure duration and by the end of the 24-h observation period.

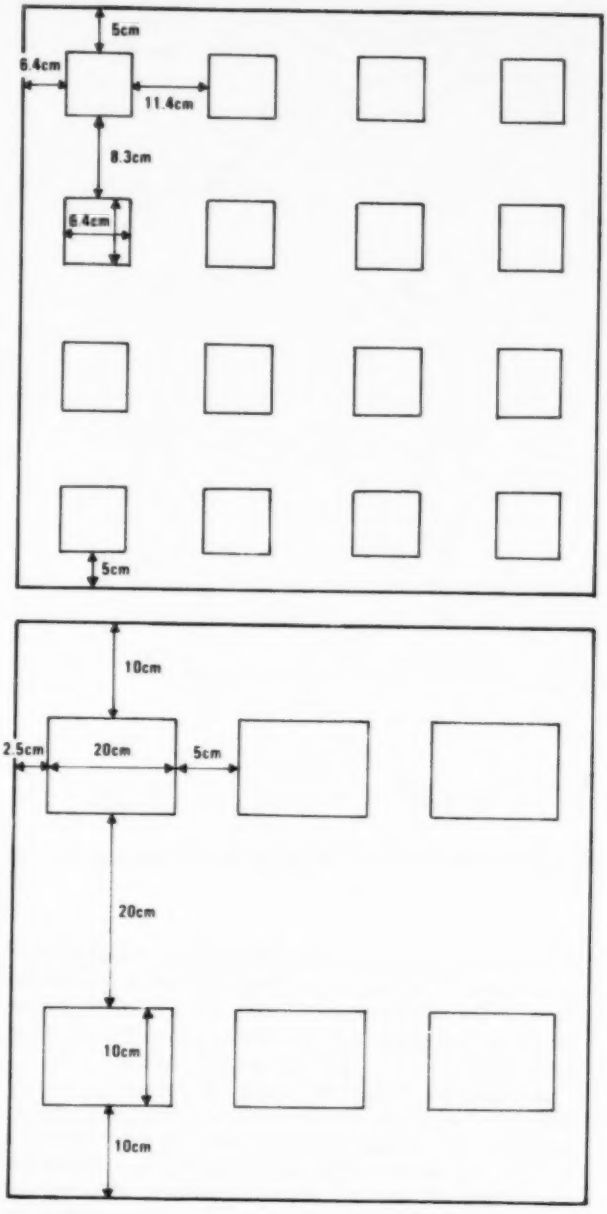

Figure 3. Dimensions of the exposure arrays for (top) 16 mice or (bottom) 6 rats used in this study.

Mice

Categorical or dummy variables for NPD, exposure duration, environmental temperature and relative humidity, and their first order interactions, were initially included in the probit analysis of variance model. Each interaction term was dropped in the presence of all other interactions and main effects to determine its χ^2 contribution via a likelihood ratio test. Since none of the interactions were significant, they were dropped from the model, and each main effect was tested in a similar fashion. The results of these tests appear in Table 2.

Since only NPD, temperature and exposure duration were significant in the analysis of variance probit model, the multiple regression probit model was confined to these variables. We found that a model using the logs of these three variables provided a very good fit to the observed data. In the form of Eqn (2), this model expresses the probability of a mouse dying from a linear combination of these variables as:

$$P = \phi[-54.18 + 5.49\ \ln(\text{PD}) + 10.70\ \ln(\text{TEMP}) + 1.04\ \ln(\text{EXPT})] \quad (3)$$

where PD is the nominal power density (mW cm^{-2}), TEMP is the environmental temperature (°C) and EXPT is the exposure duration (h).

The fit of this model to the observed data is illustrated in Fig. 4 over the tested NPD range for each of the four combinations of temperature and exposure duration used in the study. Table 3 contains the estimates and 95% confidence limits of the power density LD_{50}/24 h for each of these same combinations. Table 4 shows the actual versus model predicted proportions dying for each of these groups.

Rats

Applying the same analytic approach as outlined for mice to the rat, the data resulted in the χ^2 analysis of variance breakdown shown in Table 2. For rats, there was an indication of an environmental temperature by exposure duration interaction [$\Pr(\chi_1^2 > 3.84) = 0.05$] in addition to the main effects of NPD, environmental temperature and exposure duration. A multiple regression probit model including these four terms was then fit to the data. Once again, logarithms of these variables provided a good fit to the observed proportions. The probability of a rat dying under these exposure conditions can be expressed as:

$$P = \phi[-34.07 + 3.67\ \ln(\text{PD}) + 5.84\ \ln(\text{TEMP}) + 1.92 \times \ln(\text{EXPT}) - 0.43\ \ln(\text{TEMP})\ \ln(\text{EXPT})] \quad (4)$$

where PD, TEMP and EXPT are the same as in Eqn (3).

The fit of the above model to the observed data for rats is shown in Fig. 5 over the tested power density range. Each curve represents the form of the model for each of four combinations of temperature and exposure time used in the study. Tables 3 and 4 contain the same data for rats as presented for mice.

Single-animal vs multiple-animal exposures

An additional experiment was conducted to see if the mortality experience for animals exposed one at a time differed from that of animals exposed in groups. A total of 32 mice, similar to those used in the main study, were exposed singly to 25 or 50 mW cm^{-2} in the center of the field for 1 h at 20 °C and 80% relative humidity. These data were compared to that generated under similar conditions in multiple-animal exposures of the main experiment. Using the probit analysis of variance techniques described earlier, Table 5 indicates no effect due to the type of exposure or due to the interaction of NPD and type of exposure. Not surprisingly, most of the difference in the mouse mortality could be attributed to NPD. At least under the environ-

Table 1. Number of male CD-1 mice and CD rats that died from exposure to 2450 MHz circularly polarized microwaves

Nominal power density (mW cm^{-2})	Exposure duration (h)	Environmental data: Temperature (°C)	Environmental data: Relative humidity (%)	Number of animals died: Mice[a] At 24 h	Mice[a] During exposure	Rats[b] At 24 h	Rats[b] During exposure
10	1	20	35	0	0	0	0
			80	0	0	0	0
		30	35	0	0	0	0
			80	0	0	0	0
	4	20	35	0	0	0	0
			80	0	0	0	0
		30	35	0	0	0	0
			80	0	0	0	0
25	1	20	35	0	0	0	0
			80	0	0	0	0
		30	35	7	0	0	0
			80	4	0	0	0
	4	20	35	0	0	0	0
			80	0	0	0	0
		30	35	16	16	0	0
			80	16	16	1	1
50	1	20	35	6	1	0	0
			80	6	0	0	0
		30	35	16	9	3	1
			80	16	15	3	3
	4	20	35	10	10	0	0
			80	12	12	0	0
		30	35	16	16	4	4
			80	16	16	6	6
75	1	20	35	16	9	1	1
			80	14	9	2	2
		30	35	16	16	6	5
			80	16	16	6	4
	4	20	35	16	16	5	5
			80	16	16	3	3
		30	35	16	16	5	5
			80	16	16	6	6

[a] Number died of 16 mice.
[b] Number died of 6 rats.

Table 2. Contributions of various factors and interactions for analysis of variance models for mice and rats

Source	d.f.	Mice χ^2	Mice P	Rats χ^2	Rats P
Nominal power density	3	505.2	<0.0001	119.9	<0.0001
Temperature	1	152.5	<0.0001	48.4	<0.0001
Duration	1	37.3	<0.0001	5.4	0.0201
Relative humidity	1	0.4	0.5271	1.3	0.2542
Nominal power density X temperature	3	0.0	0.9999	3.9	0.2725
Nominal power density X duration	3	0.4	0.9402	3.9	0.2725
Nominal power density X relative humidity	3	2.3	0.5125	0.9	0.8254
Temperature X duration	1	0.0	0.9999	3.7	0.0544
Temperature X relative humidity	1	0.0	0.9999	1.6	0.2059
Duration X relative humidity	1	0.3	0.5839	0.0	0.9999
Residual (mice)	493	133.5	0.9999		
Residual (rats)	173			65.8	0.9999

Table 3. Nominal power density LD_{50}/24 h and corresponding 95% confidence limits for each combination of exposure duration and environmental temperature, for mice and rats

Exposure duration (h)	Environmental temperature (°C)	LD_{50} (24 h) (mW cm^{-2})	96% Confidence limits	
			Lower	Upper
		Mice		
1	20	56	52	61
1	30	25	23	28
4	20	43	39	48
4	30	20	17	23
		Rats		
1	20	92	74	114
1	30	48	41	57
4	20	72	61	86
4	30	40	34	49

mental conditions imposed in this supplementary experiment, it seems reasonable to conclude that exposing the mice singly or in groups of 16 does not affect the 24-h mortality rate.

DISCUSSION

There have been a fair number of experimental attempts to define the potential to kill during or immediately after exposure to RF radiation. Table 6 is a summary of 20 reports which deal with this definition. Most of the reports

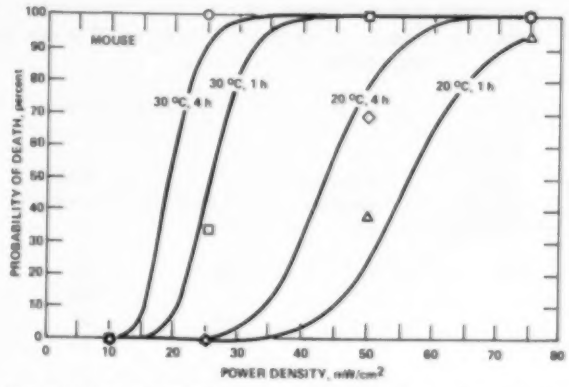

Figure 4. Fit of nominal power density model to lethality data of mice.

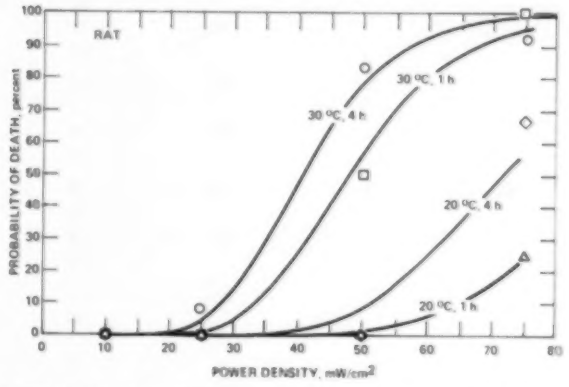

Figure 5. Fit of nominal power density model to lethality data of rats.

Table 4. Observed and model-predicted lethality rates for combinations of exposure duration, environmental temperature and nominal power density

Exposure duration (h)	Environmental temperature (°C)	Nominal power density (mW cm^{-2})	Proportion dead		Predicted rate	
			Mice[a]	Rats[b]	Mice[a]	Rats[b]
1	20	10	0.0000	0.0000	0.0000	0.0000
		25	0.0000	0.0000	0.0000	0.0000
		50	0.3750	0.0000	0.2621	0.0129
		75	0.9375	0.2500	0.9441	0.2296
1	30	10	0.0000	0.0000	0.0000	0.0000
		25	0.3438	0.0000	0.4578	0.0082
		50	1.0000	0.5000	1.0000	0.5056
		75	1.0000	1.0000	1.0000	0.9484
4	20	10	0.0000	0.0000	0.0000	0.0000
		25	0.0000	0.0000	0.0014	0.0001
		50	0.6875	0.0000	0.7911	0.0885
		75	1.0000	0.6667	0.9988	0.5056
4	30	10	0.0000	0.0000	0.0001	0.0000
		25	1.0000	0.0833	0.9101	0.0384
		50	1.0000	0.8333	1.0000	0.7823
		75	1.0000	0.9167	1.0000	0.9884

[a] $n = 32$.
[b] $n = 12$.

Table 5. Analysis of variance nominal of power density and type exposure (single vs multiple-animal) in mice

Source	d.f.	χ^2	p
PD[a]	1	21.4	<0.0001
TYPEX[b]	1	0.1	NS[c]
PD X TYPEX	1	0.0	NS
Residual	60	43.1	NS

[a] PD = Nominal power density (25 or 50 mW cm^{-2}).
[b] TYPEX = Type of exposure (single or multiple-animal).
[c] NS = not significant.

estimate the LD_{50} (at various times during and after exposure) by relating applied power densities and fixed durations of exposure. Some of these experiments also include variables such as RF frequency,[9] body weight of the irradiated subject,[10–12] sex,[12,13] or environmental temperature.[14] Phillips *et al.*[15] related death incidences to variables of SAR (and power density), body weight (average absorbed dose), environmental temperature and near- vs. far-field conditions. The difficulties of attempting to relate studies of only individual experimental variables to the biological effect (such as lethality) is seen in the review of thermal effects by Michaelson.[36] In this paper, the author could not make statistically supported conclusions on the degree of influence the employed experimental variables had on the degree of effect. That is because these reports do not contain statistical estimates of the degree of contribution that each experimental variable made to the demise of the experimental subjects.

One of the expected results of this study was the ability to determine just that. By using more sophisticated and modern statistical analytical techniques, we were able to determine, for example, the insignificant influence that relative humidity (RH) had on survival after exposure to microwaves. Before this, two analytical approaches were available. First, one could determine the incidence of lethality using a variety of relative humidities with all other experimental conditions (environmental temperature, power density, etc.) remaining the same throughout, and relating the survival to RH using probit analyses. These analyses have been used in determining LD_{50} responses to environmental temperature, for example, by a number of investigators.[11,14,15] The disadvantage of this approach is that statistical relatability is limited to the incidence of lethality and the one variable (environmental relative humidity).

A second approach to solving the problem of relating a number of variables influencing RF-radiation-induced lethality is to combine variables mathematically on some pre-determined basis. The Temperature-Humidity Index (THI) is one such combination. The THI [defined as THI = (1.44 °C + 0.1 RH + 30.6)] was used by Rugh *et al.*[16] as an aid in determining the lethal-response of mice to 2450 MHz microwaves. The THI in that study ranged from 55 to 95; the range of temperatures of the environmental air was 15–40 °C and the RH 25–70%. However, the THI, originally introduced by Mumford[17] to the field of RF radiation standards, is used as an expression of human comfort. There are no experimental data using objective criteria

Table 6. Summary of published reports that include data on RF radiation-induced lethality (Checkmarks indicate variables in the study)

Species	Power density	SAR	Fixed durations	Time-to-death	LD_{50} estimate	Other effects studied	Ref.
Rats	X		X		X		18
	X		X				23
	X		X		X	Frequency	9
	X		X		X		24
	X		X		X		25
	X		X		X	Interval between irradiations	26
	X	X	X		X	Body weight	10
						Near- vs far-field	15
	X			X		Body weight	11
			X	X		Environmental temperature; oxygen in air	14 27
	X	X	X		X	Environmental temperature; RH	This
Mice	X		X		X		19
	X		X				23
	X		X		X		28
			X	X	X		29
	X			X			30
		X	X	X		Sex; THI	16
				X		Sex; body weight	12
						Cholinergics	31
	X	X	X		X	Environmental temperature; RH	This
Guinea pig	X		X				32
Dog			X	X		Body weight (only head irradiated)	33

that confirm its application to animals. The use of the THI in rodents seems especially inappropriate given the lack of skin sweating in these animals. There is no widely accepted analog of the THI for use in rodents.

In this study, the THI ranged from 63 to 82. If RH is not included in the THI calculation, and a 'temperature index' is calculated instead, the range is only decreased to 60–74. This is because the scale factor for RH in the THI is only 0.1 and the entire possible range of RH (0–100) could contribute only a maximum of ten to the THI. Therefore, the relative humidity is expected to contribute little to the death rate.

Experimentally, we found very little influence of the RH on the rate of death in earlier studies on mice or rats. The X^2 contributions due to RH from the probit analysis of variance models in either mice or rats (Table 2) do not approach significance. One wonders about the continued need to enforce rigid control of RH in other acute studies of RF-radiation-induced biological effects in whole animals, especially in rodents in environmental temperatures not above 30 °C.

However, environmental temperature contributed significantly to death in this study. The LD_{50} value decreased greatly in 1- and 4-h exposure situations in both mice and rats when environmental temperature was increased from 20 °C to 30 °C (Table 3). The 10 °C increase resulted in a LD_{50} value approximately 50% less. Deichmann *et al.*[14] and Anzai *et al.*[11] found similar responses to increasing environmental temperatures in rats exposed to microwaves. They used 24 GHz[14] or, we suspect, 2.45 GHz[11] frequencies and observed rats for their times of death during exposure. At an environmental temperature of 30 °C, Deichmann *et al.* reported mean periods of survival of 21 min. 20 °C, the same measurement was 36 min. In the report of Anzai *et al.*, an increase from 12 to 22 °C caused a shift of mean survival time from 30 min to 12 min, a 60% decrease. Also, Samaras *et al.*[35] prevented any deaths in rats exposed to usually lethal levels of 2.45 GHz microwaves just by blowing nitrogen-cooled air over them.

This measurement of period of survival during irradiation is philosophically different than the measurement we used. The incidence of death at 24 h after the beginning of irradiation. However, we did count the number of mice and rats which were found dead at the end of the irradiation period in this study. Comparisons of the incidences of rats that died during the exposure at 20 or 30 °C as percentages of those that died by 24 h are not feasible, because all but four rats died during exposure. Of the mice that died after a 1-h exposure at 20 °C, 45% (19/42) died during exposure. At 30 °C, 75% (56/75) died during the 1-h exposure, an increase of 1.7-fold. In the probit analyses of variance, exposure duration was a very significant ($p < 0.0001$) contribution to the death rate in the mouse and marginally significant ($p = 0.02$) in the rat (Table 2). The increase of exposure duration from 1 to 4 h resulted in a decrease of approximately 20% of the LD_{50} value at equal temperatures in both species.

That duration of exposure, at least within the range used in this study, is a significant factor in the lethality of RF radiation is confirmed by other studies. Polson *et al.*[9] exposed rats at a number of frequencies, power densities and exposure durations, and calculated LD_{50}'s for each exposure duration-power density combination. A plot of these LD_{50}'s shows that the probability of death at any power density is related to duration and that death tends to occur early in an exposure situation. Tikhonchuk and coworkers also showed a similar relationship between death and the length of exposure to another series of power densities in rats[18] and mice.[18,19]

In this study, the range of nominal power densities was chosen to obtain combinations with exposure duration and environmental temperature expected to cause a range of lethality from zero to almost 100%. It was not possible to do this for both species, as the mouse absorbs approximately 2–4 times more radiation at this frequency than the rat.[20] This is seen in Fig. 5, in which the predicted death rates for rats exposed at 20 °C do not approach 100% at the highest NPD tested. An expected power density to kill almost all rats exposed for 1 h at 20 °C cannot be confidently estimated from the data collected in this study.

However, some prediction can be made for the probability of death from lower doses. From Eqn (3), approximately 46% of mice would be expected to die when exposed to 20 mW cm^{-2} for 4 h at 30 °C. At 15 mW cm^{-2} approximately 7% would be expected to die. Mice prefer ambient temperatures of 31–31.5 °C.[21] The low temperature used in this study was considerably below this comfort range. However, exposure of mice at 20 °C to 15–20 mW cm^{-2} (SAR = 19.4–13.8 W kg^{-1}) for 4 h still represented a considerable deposition of energy and resulted in a significant probability of death.

Of the studies listed in Table 6, two were conducted using anechoic chambers in which animals were exposed one at a time, even though the available exposure area may have been relatively uniform.[9,10] There is an economy associated with exposing a large number of animals at one time, such as done in this study. However, the possibility does exist that an arbitrarily chosen exposure matrix may increase the variability of absorption and prevent a clear distinction between doses.[22] With this in mind, and with a clear need to shorten the term of the study, the choice was made to develop and use a multi-animal exposure array that produced lower variability in absorption for each animal (Fig. 3). Low absorption variability was also ensured by the form of propagation (circular polarization).

The analyses comparing the data of the experiment using single-animal exposures of mice with that of multi-animal (16 mice) exposures did not show a statistical difference. For mice at least, the combination of the use of circularly polarized propagation and the care taken in deciding on the exposure array could have influenced our inability to detect any difference in mortality between the two types of exposure methods. Other statistical analyses considered the body weight of the animals and also the position each animal occupied during exposure. Neither of these variables had a significant influence on the death rates.

When the experiment was first designed, we expected to demonstrate that a model using SAR to express the microwave dose rate would be a better predictor of mortality than a model using NPD. For that reason, a considerable effort was placed on estimating SAR values for each species in each position, by developing exposure geometries that allowed multiple-animal exposures without sacrificing too much uniformity of absorption, and by restricting the range of acceptable body weight. We also calculated SAR for each position on the basis of power density estimated from the field contour plots, as well as on the basis of nominal power density. A further check was made by exposing animals

singly, thereby avoiding the confusion arising from the random motion causing random reflections during the multiple-animal exposures. Also, we used circular polarization to cause 'stirring' of the propagated wave. However, all analyses that included estimated SAR as the dose rate variable (position, body weight, positional SAR and positional SAR corrected for calculated spot power density) were no more efficient than those which used NPD instead. We believe that the cause of this unexpected result is that there is a greater statistical error associated with SAR than with NPD, even though state-of-the-art techniques[34] were employed in both the determinations.

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